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EVALUATION OF PHARMACOLOGIC AGENTS TO SUPPRESS INTRAOCULAR CELLULAR PROLIFERATION FOLLOWING TRAUMA

FINAL REPORT

September 30, 1982 - June 15, 1986

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Block 20. In an animal model of tractional retinal detachment using both labeled and unlabeled retinal pigment epithelial cells, we determined that methyltrexate and 5-FU were the most effective currently considered pharmacologic agents for the suppression of intraocular cellular proliferation based upon suppression of clinical tractional retinal detachments.

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SUMMARY

As the limits of mechanical vitreoretinal surgical techniques are being reached, it has become clear that the main cause of lost eyes from trauma and proliferative vitreoretinopathy is due to intraocular cellular proliferation. Several agents have been suggested to control intraocular cellular proliferation. This study was designed to test these agents (5-FU, methotrexate, prostaglandin PGE1, dexamethasone, trimacinolone, indomethacin, colchicine, d-penicillamine) against control eyes as well as to test the toxicity at timing of injection.

In a rabbit model of tractional retinal detachment using retinal pigment epithelial cells as an injected cell, bolus pharmacologic agents were tested under matched conditions both early after cell injection and late after cells were allowed to divide. Clinical detachment, light microscopic evaluation of cellular proliferation, and electron microscopic evaluation of the retina for toxicity and electrophysiologic testing were all used to evaluate each drug following both early and late injection of pharmacologic agents. We found 5-FU and PGE1 to show the greatest promise for suppression of clinical detachment and lower retinal toxicity in the retinal rabbit model for early and late injection of cells respectively. These drugs attack these cells at various points in the synchronized cell life cycle in the animal model. Due to the lack of a synchronized cell cycle in the tractional detachment of trauma, it may be advisable to consider a bolus of several drugs affecting both cell division as well as cell contractility.

FOREWORD

In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Natural Research Council (DHEW publication No. (NIH) 78-23, Revised 1978).

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BODY

This report summarizes the work done between September 30, 1982 and June 15, 1986, testing several pharmacologic agents in a controlled rabbit model of tractional retinal detachment. This report is a composite of the Annual Report of 1983 and 1984 for Contract No. DAMD17-82-C-2264 and the summary of work concluded in 1985 and 1986 for Contract No. DAMD17-85-C-5007.

Essentially the first year of this study was devoted to the development of the clinical model and the early (immediately after cell injection) or late (after cells had been allowed to divide) injection of selected pharmacologic agents. These agents included 5-FU 2 mg intravitreally, methotrexate 2 mg, colchicine 50 ug, Indomethacin 1 mg, d-penicillamine 2.5 mg, dexamethasone 2 mg, triamcinolone 2 mg and prostaglandin PGE1 2 mg. These drug injected animal models were tested against control eyes and each drug was tested both early (immediately after cell injection) and late (injection one week following the injection of retinal pigment epithelial cell bolus.) In the annual report in September 1983 for Contract No. DAMD17-82-C-2264, the results of the clinical observations of these injections were reported and are again shown in this report in Tables 1-9.

At that time it was our feeling that the clinical observations supported the use of 5-FU or methotrexate as a drug of choice for immediate injection into the vitreous cavity following an injected cell bolus. We interpreted this clinically to mean that these drugs would be most helpful if injected immediately after the time of trauma. We also found at that time that prostaglandin PGE1 or d-penicillamine seemed to be an effective agent in suppressing myofibroblastic contraction or to be a drug considered for reduction of tractional retinal detachment if late injection was considered. All of the drugs in this clinical part of the study had been injected in a BSS carrier and had variable stability within this carrier, the prostaglandin being perhaps the most unstable.

We felt that one of the draw backs of our animal model was that cell synchrony was established by the fact that the cells injected had a similar time frame in regards to cell division and therefore, cell contraction. Since in the clinical setting there are probably many populations of cells going through both division and contraction at the same time, we felt that an injected pharmacologic bolus of perhaps several drugs with a broad spectrum of activity within the multiplicity of cell circumstances might be most effective although the various interactions of these drugs and the effect of this type of combination drug therapy was not studied within the scope of this project. Electrophysiologic testing was performed prior to pharmacologic injections in the rabbit eyes. It was felt by our veterinarian electrophysiologist (G.B.) that this testing early on was not providing interpretable data and therefore electrophysiologic testing was abandoned as the study progressed.

FIRST YEAR

The technique of pigment epithelial harvesting, incubation of cells, and micrographic evaluation followed by surgical implantation of cells has been described in the clinical results presented in Tables 1-9 of the 1983 Annual Report for Contract No. DAMD17-82-C-2264.

SECOND YEAR

During the second year of this study following harvesting of eyes, they were studied in multiple sections by light microscopy to determine the comparative efficacy between control and drug injected eyes in regard to suppression of cellular proliferation and cellular attachment along the epiretinal surface. Eyes were removed in the control group and animals sacrificed at one week. The other animals were sacrificed and eyes harvested at two weeks, four weeks, six weeks, and three months. Eyes removed were then fixed opened and studied grossly by dissecting microscopy to reconfirm the clinical impression of epiretinal proliferation and tractional detachment. These eyes had previously been fixed in gluteraldehyde.

Following gross examination, representative areas were taken for thick section light microscopic study and electronmicroscopy, both transmission and scanning. Each eye had multiple samplings through the medullary ray and periphery as well as other areas depending on their gross appearance. For each drug one eye (two week eye) was taken for whole eye study with light microscopy to determine regional variation in epiretinal proliferation as well as generalized cellular integrity as a test for drug toxicity as has been summarized in the annual report of the second year's work, April 1986 Contract No. DAMD17-85-C-5007.

Gross ocular examination demonstrated extensive tissue damage to be present with the use of colchicine and methotrexate. Our previous work has demonstrated that colchicine in lower dose may have less of a tissue destruction. This toxicity appears to be based on its effect on the microtubular system of all cells but in the dose tested here tissue destruction is indeed extensive.

Methotrexate, which seems very promising initially, clinically, showed extensive destruction of the outer retina which seem to correlate to a drug toxicity. Interestingly, this drug showed a blanching of the retina at gross examination commented on by two observers.

D-penicillamine, which initially showed promise in regards to clinical studies also showed alteration in cell morphology suggesting a retinal toxic effect even at the light microscopic level. Prostaglandin PGE1 did seem to be well tolerated in the eye, not showing light microscopic alteration.

With microscopy, the numbers of cells and the rate of cell-to-cell attachment was examined. In our second annual report, April 1986, light microscopic data on d-penicillamine and indomethacin was not available. Since that time, light microscopic studies have yielded information showing a toxic effect of the d-penicillamine on retinal cells showing cellular destruction in areas where drug appeared to have accumulated. It also showed in the later injected eyes, one week following cell bolus injection, that the cells were there in large number but, that the observation of what appears to be cell destruction, even at the light microscopic level, would deter us from suggesting this as a clinical therapy.

The indomethacin eyes, which showed extensive retinal detachment, had very poor views on clinical examination. In some sections, bacteria were present suggesting that some of the results in the indomethacin group may have been colored by an overriding endophthalmitis. We did not have drug for culture still available at the time that this correlation was discovered.

THIRD YEAR

The third year of our study was devoted to electronmicroscopy. This part of the report will be divided into sections on 5-FU, methotrexate, prostaglandin PGE1, dexamethasone, triamcinolone, indomethacin, colchicine and d-penicillamine. We will attempt in each of these sections to correlate the clinical appearance, light microscopic appearance, and electronmicroscopic appearance of the rabbit eyes.

5-FU

5-FU, which was used in 2 mg intravitreal injections, clinically, showed a good effect in our early injection group. On light microscopy, the cells injected in the early group tended to not form sheets of epiretinal membrane, but were isolated in islands of individual cells both in areas of vascular retina predominantly (medullary rays), but also in areas of nonvascular retina. In the later injected groups, tractional retinal detachment was more prominent. Also, the cells themselves tended to appear more commonly in sheets along the epiretinal surface suggesting that cell proliferation had continued. Electronmicroscopic study of 5-FU showed retention of retinal cell structure in areas of avascular retina, also the vasculature in the medullary ray appeared to be intact. (Figure 1a) The inner retinal surface also appeared unaltered in these eyes injected with 2 mg of 5-FU intravitreally. (Figure 1b & 1c) Considering the clinical efficacy of 5-FU and lack of retinal toxicity, this does seem to be a very effective agent, especially with early injection of the drug. Its injection at a later date seems to be less effective. 5-FU, however, might seem to be a very good agent to suggest when coupled with surgical therapy since at that time, synchrony of the intraocular cell bolus may be more closely achieved. This synchrony of cell division may make 5-FU a more effective drug. It also may be that repeated injection or prolonged release may be helpful until the cells go into an inactive phase.

Methotrexate

Methotrexate was used as a 2 mg intravitreal injection. It was found to be quite effective when injected early with the five eyes injected not showing retinal detachment and maintaining a relatively clear media. The late injected eyes showed two out of five with corneal haze early on and two out of five went on to form tractional retinal detachment. Methotrexate was one of the most surprising results showing an extensive area of retinal cell destruction. This we found to be confirmed with electronmicroscopic study showing a retinal cell destruction, particularly the outer layers, and certainly deters us from considering methotrexate as a useful clinical modality in this setting. (Figure 2a & 2b)

Colchicine

Clinical examination of colchicine injected eyes with 50 ug in the vitreous cavity showed orange reflexes initially in 7 of the 10 injected eyes both early and late. These eyes eventually showed no view with three eyes showing no view initially. The gross and light microscopic examination showed retinal necrosis and the electronmicroscopic examination confirmed extensive retinal necrosis and destruction of

TABLE 1

CONTROLS

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
1	2	C	+	/ S A C R I F I C E D /				+
2	1	C	+	+	//	//	//	+
3	2	C	+	+	+	//	//	+
4	2	C	+	+	+	+	//	+
5	Blood	C	N O V I E W					Total detach. Vit. bleed
One week later injection of drug								
6	2	C	+	//	//	//	//	+
7	Corneal Haze 3	C	+	+	//	//	//	+
8	2	C	+	+	+	//	//	+
9	1	C	+	+	+	+	//	+
10	3	C	+	+	+	+	+	+

Table shows the clinical results of control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* **Clinical Detachment included puckering of medullary ray and peripheral detachm weeks after drug injection**

** **Done following enucleation**

Figure 1a

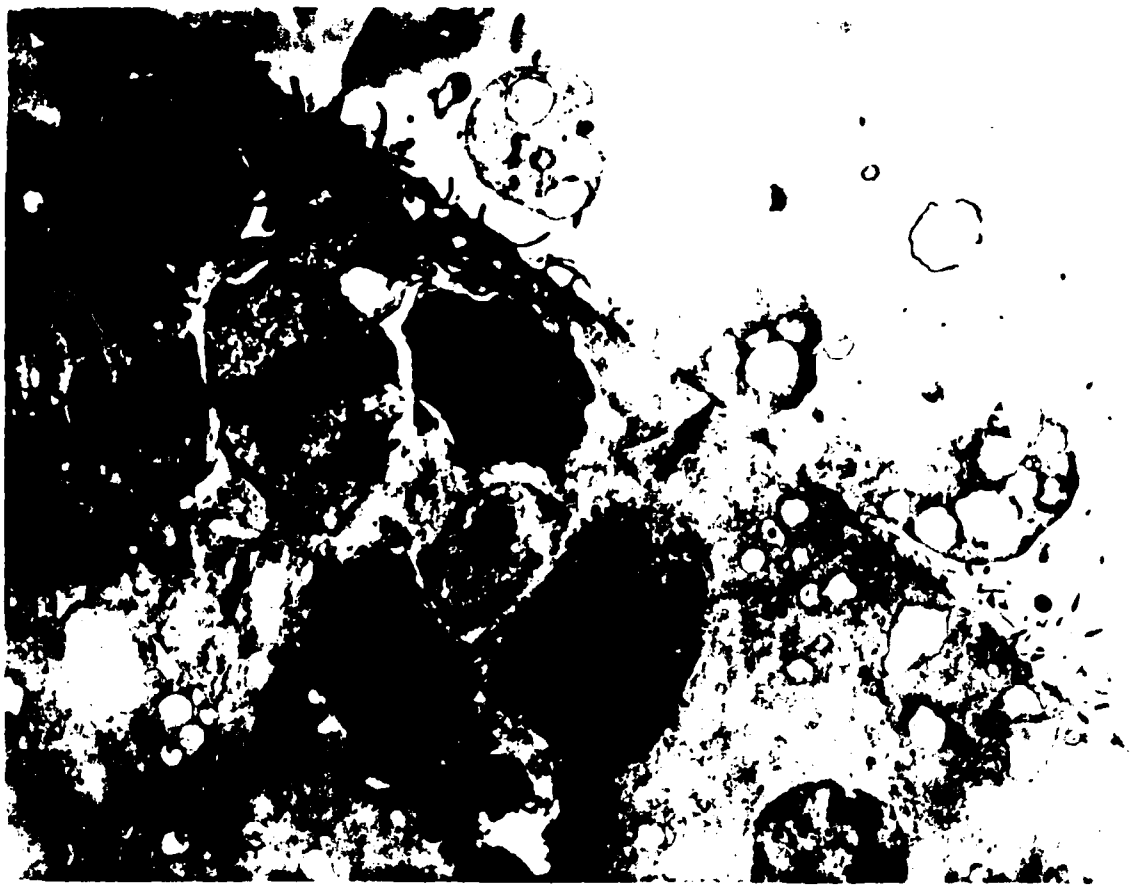


Figure 1a - Electronmicrograph showing retinal surface with cells attaching to retinal surface although without cell to cell attachment. In some areas, cells appear to be arising from the retinal surface. In some areas attachment plaque appears present. (3600x)

Figure 1b

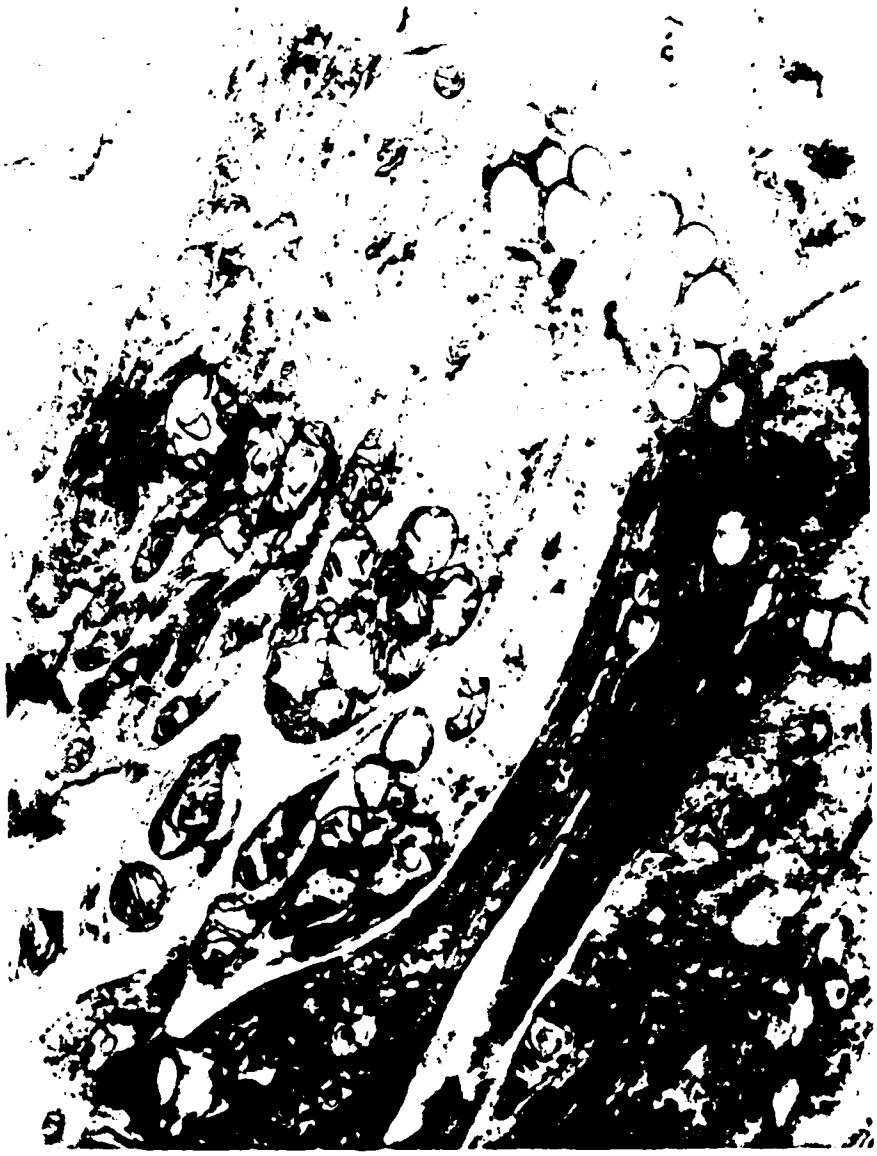


Figure 1b - Electronmicrograph showing preserved sensory retinal structure. (5900x)

Figure 1c

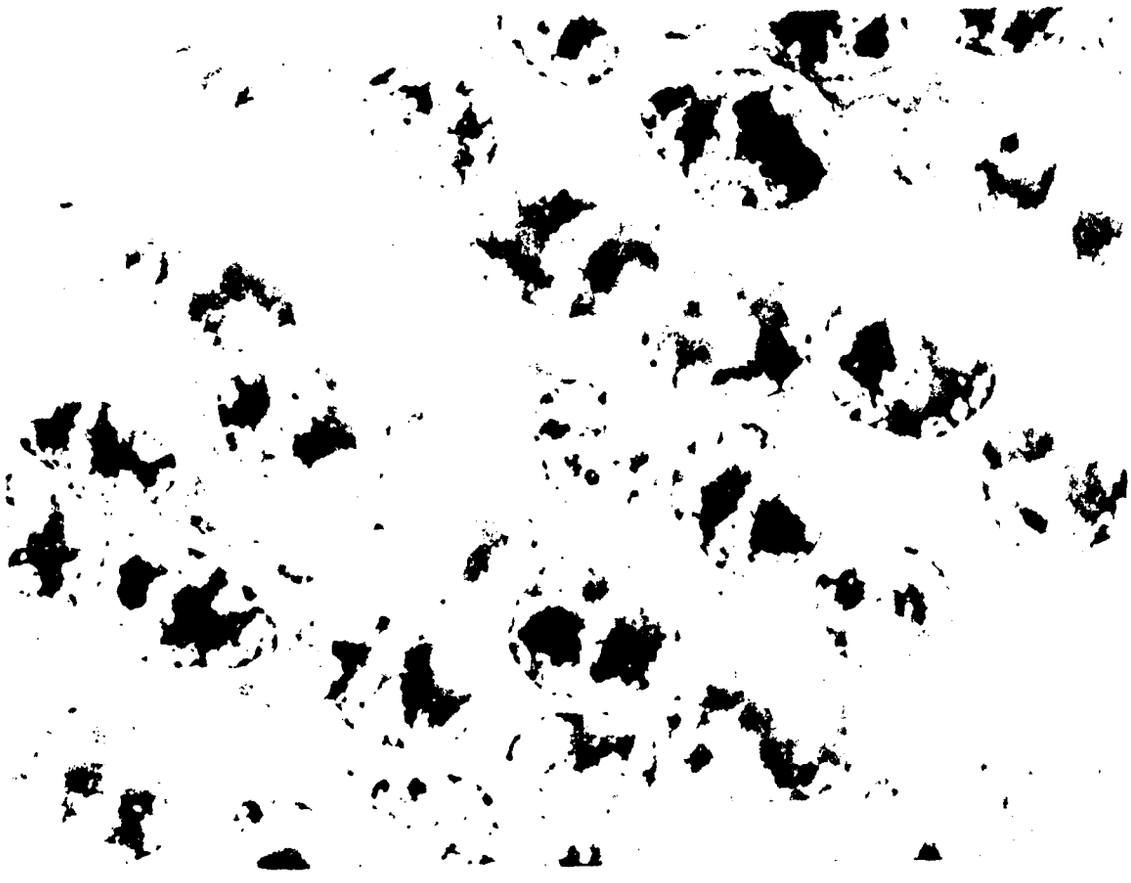


Figure 1c - Electronmicrograph showing the retained structure on a fonte section of nuclear retinal layer. (2600x)

TABLE 2

5-FU 2 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
11	3	5-FU	-	/ S A C R I F I C E D /				-
12	1	5-FU	Corn. Haze	-	//	//	//	-
13	1	5-FU	-	-	-	//	//	-
14	1	5-FU	Corn. Haze	Corn. Haze	-	-	//	-
15	2	5-FU	Corn. Haze	-	+	+	+	+ Retinal Hole
One week later injection of drug								
16	2	5-FU	Corn. Haze	//	//	//	//	-
17	2	5-FU	-	-	//	//	//	-
18	2	5-FU	+	+	+	//	//	+
19	3	5-FU	+	+	+	+	//	+
20	3	5-FU	-	-	-	-	-	-

Table shows the clinical results of 5-FU against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

*** Clinical Detachment included puckering of medullary ray and peripheral detachment weeks after drug injection**

**** Done following enucleation**

TABLE 3

METHYLTREXATE 2 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
21	2	M	-	/ SACRIFICED /				-
22	1	M	-	-	//	//	//	-
23	3	M	-	-	-	//	//	-
24	1	M	-	-	-	-	//	-
25	1	M	-	-	-	-	-	-
One week later injection of drug								
26	3	M	No View	//	//	//	//	-
27	2	M	Corn. Haze	-	//	//	//	-
28	1	M	-	-	+	//	//	+
29	2	M	Corn. Haze	NO	VIEW		//	-
30	1	M	NO	VIEW				+

Table shows the clinical results of methyltrexate against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* **Clinical Detachment included puckering of medullary ray and peripheral detachme weeks after drug injection**

** **Done following enucleation**

Figure 2a

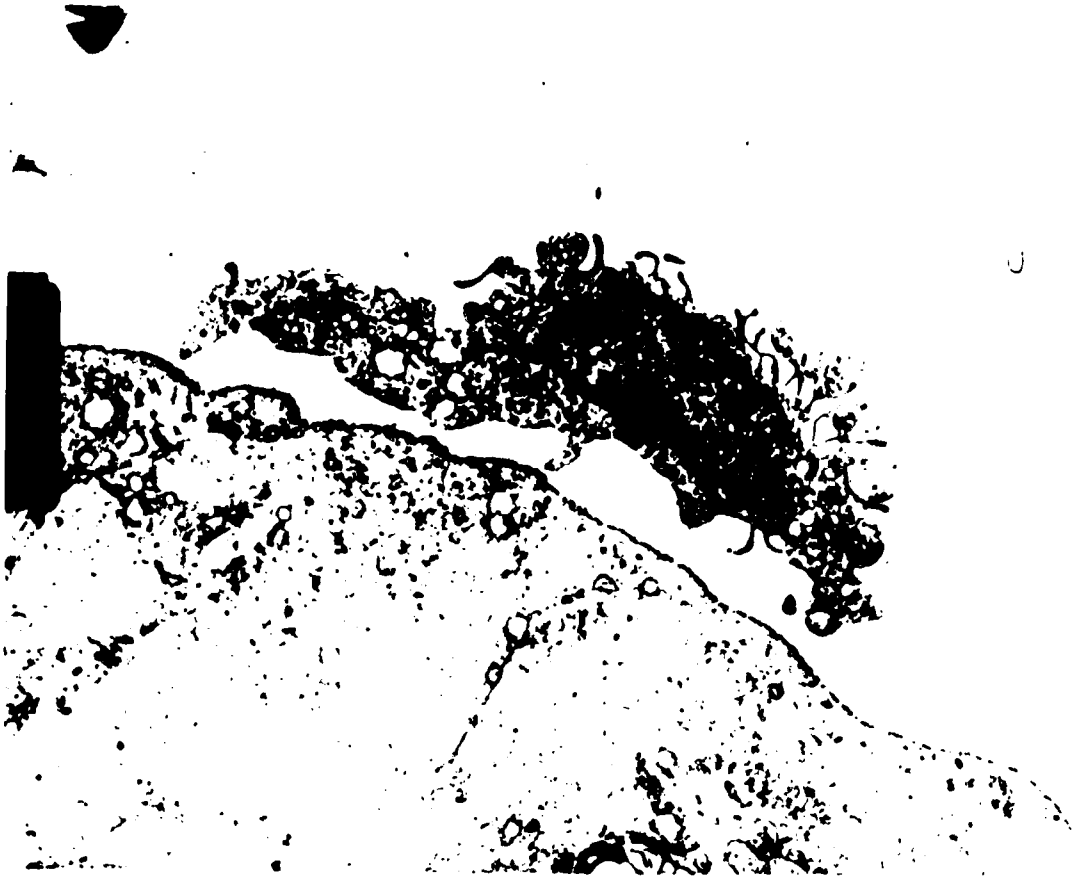


Figure 2a - Electronmicrograph showing injected cell along retinal surface (medullary ray). Cells not found in sheets but tended to be individual cells. (2600x)

Figure 2b

Figure 2b - Shows outer segments in area of attached retina which have lost grossly regular pattern and in some areas loss of disc organization. (5900x)

tissue, presumably due to the cytotoxic effect of the microtubular system as a whole. Our previous work on colchicine showed that the therapeutic dose is very close to the toxic dose. In previous pilot studies, colchicine at the 100 ug level was shown to be retinal toxic but at 10 ug seemed to have some positive effects. In a recent personal communication, Glaser feels that colchicine in oral dose in animal model may generate some reduction in tractional detachments due to its microtubular toxic effect, however, in this oral administration, it is not known what the intravitreal dose might be. It may be that the very low effective dose is possible to achieve in the vitreous cavity with oral administration. Although, certainly the toxic dose is very close to the therapeutic dose in this drug. This drug may merit further evaluation over a wide dosage range and administration routes. (Figure 3)

Indomethacin

1 mg suspension was injected into the vitreous cavity of the animals of the tractional retinal detachment model. We found that in 9 of the 10 animals injected with indomethacin no view was achieved at one week and one of the animals died an anesthetic death. The light microscopic studies suggested an endophthalmitis with contamination of the indomethacin. This indomethacin, unfortunately, was discarded by the time such a correlation could be made so culture was not possible to prove that the indomethacin was the contaminating cause, although based on the clinical observations and light microscopic observations, this seems a reasonable conclusion. We, therefore, are unable to conclude if indomethacin seems to be a viable pharmacologic alternative to suppress intraocular cellular proliferation or cellular contraction.

D-penicillamine

2.5 mg was injected into the vitreous cavity. The first week, clinical examinations showed no view to be present in all ten animals, however, in the gross examination of the late injected animals, three of the five eyes, although no view had been restricted by anterior segment haziness, the retina was attached in three of the five late examined eyes. On light microscopic examination, however, in these eyes extensive retinal destruction was noted. This combination of anterior segment haziness, despite monitoring of intraocular pressures coupled with the extensive retinal damage in this group, lead us to feel that this is not the drug that we would suggest for intravitreal use, despite the initial feeling that this may have represented a drug that might be useful to reduce the effects of cell to cell attachment. (Figure 4a and 4b)

Dexamethasone

Dexamethasone 2 mg was injected intravitreally and revealed clinically quiet eyes. These eyes, on gross examination, showed a majority of eyes which achieved retinal detachment with late injection, although two of the eyes showed no tractional detachment with early injection. One animal died postoperatively and two other eyes showed high tractional detachment visible both on clinical and gross examination. Light microscopic studies showed that dexamethasone was tolerated very well intravitreally showing in the early eyes no evidence of inflammatory cells as might be expected with this steroidal compound. However, inflammatory cells were not large in amount with other compounds. The eye did tolerate this quite well, but our study did not confirm a reduction in tractional detachment when compared against control eyes. Our electronmicroscopic studies confirmed that the retinal anatomy was well maintained in the face of dexamethasone injection. (Figure 5)

TABLE 4

COLCHICINE 50 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
31	3	C	Orange Reflex	/ S A C R I F I C E D /				Retinal Necrosis
32	3	C	Orange Reflex	No View	No View	//	//	Retinal Necrosis
33	3	C	Orange Reflex	No View	No View	//	//	Retinal Necrosis
34	3	C	Orange Reflex	N O	V I E W		//	Retinal Necrosis
35	3	C	Orange Reflex		N O	V I E W		Retinal Necrosis
One week later injection of drug								
36	3	C	Orange Reflex	/ S A C R I F I C E D /				Retinal Necrosis
37	2	C	Orange Reflex	No View	//	//	//	Retinal Necrosis
38	2	C	N O	V I E W		//	//	Retinal Necrosis
39	3	C		N O	V I E W		//	Retinal Necrosis
40	2	C			N O	V I E W		Retinal Necrosis

Legend: Table shows the clinical results of colchicine against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

* Clinical Detachment included puckering of medullary ray and peripheral detachme weeks after drug injection

** Done following enucleation

Figure 3

Figure 3 - Electronmicrograph shows retinal cellular destruction following intravitreal injection of colchicine. (3600x)

TABLE 5

INDOMETHACIN 1.0 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
41	3	I	No View	/ S A C R I F I C E D /				+
42	3	I	No View	+	//	//	//	+
43	4	I	N O	V I E W	+	//	//	-
44	3	I	No View	+	+	+	//	+
45	3	I	No View	+	+	+	+	+
One week later injection of drug								
46	A N E S T H E T I C			D E A T H				
47	3	I	No View	//	//	//	//	+
48	3	I	N O	V I E W	//	//	//	+
49	3	I	No View	+	+	+	//	+
50	4	I	No View	+	+	+	+	+

Table shows the clinical results of indomethacin against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* **Clinical Detachment included puckering of medullary ray and peripheral detachm weeks after drug injection**

** **Done following enucleation**

TABLE 6

D-PENICILLAMINE 2.5 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
51	3	DP	No View	/ SACRIFICED /				+
52	3	DP	NO	VIEW //		//	//	-
53	2	DP	NO VIEW			//	//	Coagulation Necrosis
54	3	DP	NO	VIEW +		+	//	+
55	3	DP	No View	±	+	+	+	+
One week later injection of drug								
56	3	DP	No View	/ SACRIFICED /				+
57	2	DP	No View	±	//	//	//	-
58	3	DP	NO VIEW			//	//	-
59	3	DP	No View	±	-	-	//	-
60	3	DP		NO VIEW			+	+

Table shows the clinical results of d-penicillamine against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* **Clinical Detachment included puckering of medullary ray and peripheral detachme weeks after drug injection**

** **Done following enucleation**

Figure 4a

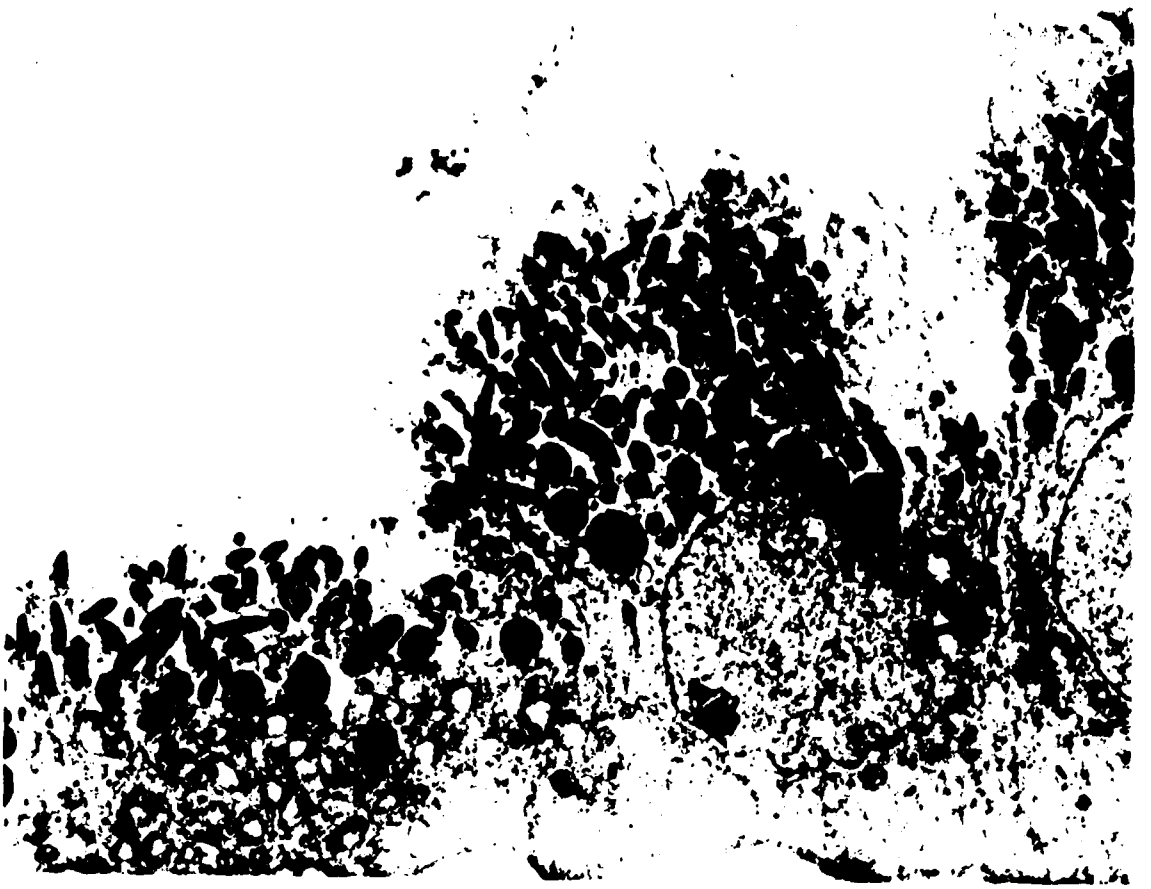


Figure 4a - Electronmicrograph showing cells with retinal pigment epithelial morphology and cell to cell junction in eyes with d-penicillamine injected. (arrow)

Figure 4b

Figure 4b - Electronmicrograph showing extensive retinal cell destruction with distended mitochondria.

TABLE 7

DEXAMETHASONE 2 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
61	1	D	-	/ S A C R I F I C E D /				-
62	1	D	-	-	//	//	//	-
63	1	D	-	-	+	//	//	+
64	D I E D P O S T		OP					
65	1	D	-	+	+	+	+	+
One week later injection of drug								
66	2	D	+	/ S A C R I F I C E D /				+
67	3	D	+	+	//	//	//	+
68	3	D	-	-	-	//	//	-
69	2	D	+	+	+	+	//	+
70	3	D	+	+	+	+	+	+

Table shows the clinical results of dexamethasone against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* **Clinical Detachment included puckering of medullary ray and peripheral detachme weeks after drug injection**

** **Done following enucleation**

Figure 5

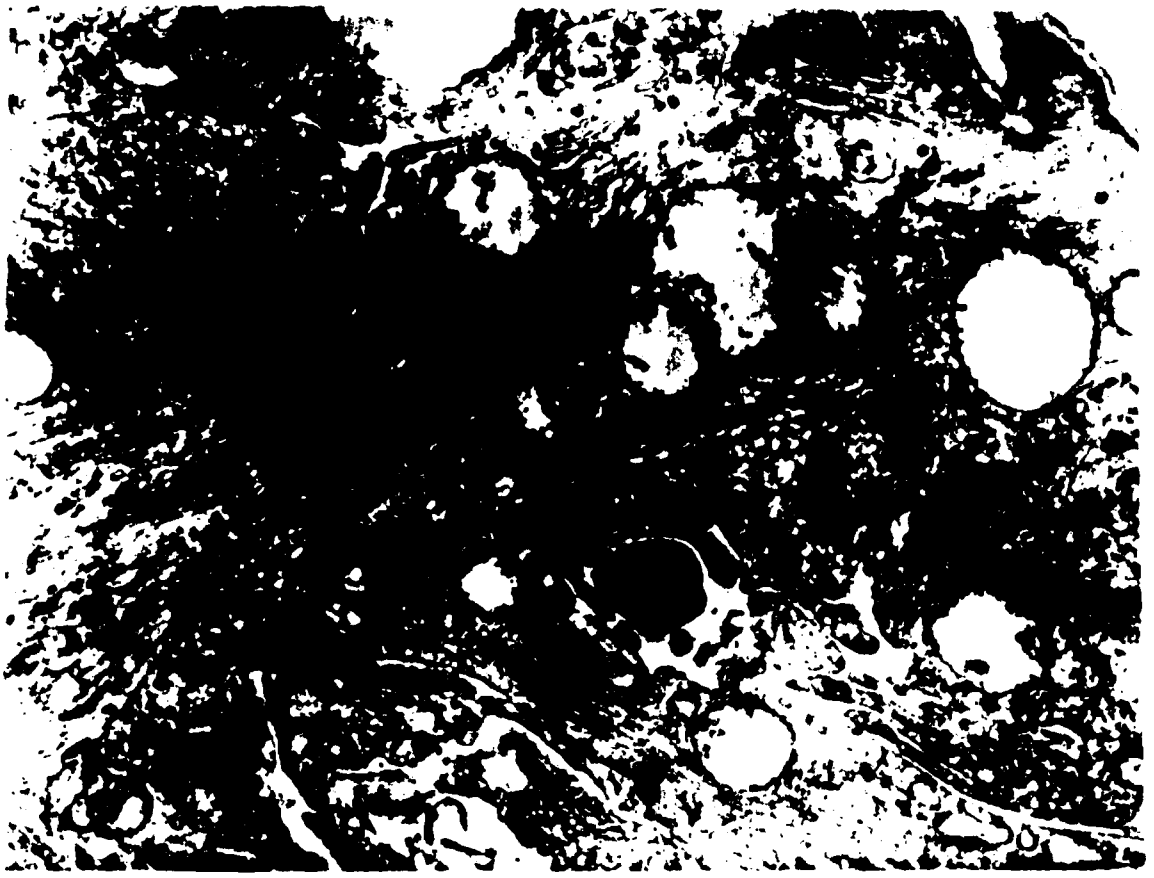


Figure 5 - Electronmicrograph showing irregular vitreal retinal surface (upper right corner) with retained retinal structure after injection of dexamethasone.

Triamcinolone

2 mg was injected into the vitreous cavity of animals who had been injected in the fashion for tractional retinal detachment model. The effects of triamcinolone seemed very comparable to dexamethasone with an inconsistent effect in the reduction of tractional retinal detachment, although the eye itself remained quiet due to its anti-inflammatory effect. We also did not find this drug to be a drug that we felt was showing strong evidence of suppression of tractional detachment. (Figure 6a, 6b and 6c)

Prostaglandin PGE1

Prostaglandin PGE1 was injected in a 2 mg suspension into the vitreous cavity. Prostaglandin is a very unstable compound, and we assume has a very brief half-life in the vitreous cavity. Early injection of the drug did not seem to be effective in terms of tractional retinal detachment. With late injection, three of five eyes showed no tractional detachment. The media remained clear. Light microscopic studies showed cells to be present in sheets, but we presumed that the prostaglandin PGE1 acted to reduce tractional detachment by reducing the effect of cellular contraction. The electronmicroscopic studies showed the retinal architecture to retain a normal appearance without microscopic destruction being present. (Figure 7a and 7b)

TABLE 8

TRIAMCINOLONE 2 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
71	0	T	-	/ SACRIFICED /				-
72	0	T	-	-	//	//	//	-
73	0	T	-	+	+	//	//	+
74	0	T	-	-	+	+	//	+
75	0	T	-	+	+	+	+	+
One week later injection of drug								
76	2	T	+	/ SACRIFICED /				+
77	3	T	+	+	//	//	//	-
78	2	T	+	+	+	//	//	+
79	1	T	-	-	-	-	//	-
80	2	T	+	+	+	+	+	+

Table shows the clinical results of triamcinolone against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* Clinical Detachment included puckering of medullary ray and peripheral detach weeks after drug injection

** Done following enucleation

Figure 6a

Figure 6a - Electronmicrograph showing tractional retinal detachment in area of medullary ray. Retina shows mitochondial loss which appears to be a fixational artifact. (2600x)

Figure 6b

Figure 6b - Electronmicrograph showing good photoreceptor outer segments and mitochondria.

Figure 6c

Figure 6c - Electronmicrograph showing heavy cellular and collagen membrane. The cells seen contain pigment.

TABLE 9

PROSTAGLANDIN PGE1 2 mg

animal #	2 day post op uveitis (1-4)	pharm. agent or control	Clinical Tractional Detachment*					Gross Exam**
			1 wk	2 wk	4 wk	6 wk	12 wk	
Immediate injection of drug								
81	2	-	-	/ SACRIFICED /				-
82	1	P	-	+	//	//	//	-
83	2	P	-	+	+	//	//	+
84	1	P	+	+	+	+	//	+
85	ANESTHETIC DEATH							
One week later injection of drug								
86	2	P	-	/ SACRIFICED /				-
87	3	P	+	+	//	//	//	+
88	3	P	+	+	+	//	//	+
89	2	P	-	-	-	-	//	-
90	3	P	-	-	-	-	-	-

Table shows the clinical results of prostaglandin PGE1 against control eyes, clinical complication, and clinical versus gross pathologic exam of eyes.

Legend:

* Clinical Detachment included puckering of medullary ray and peripheral detachm weeks after drug injection

** Done following enucleation

Figure 7a

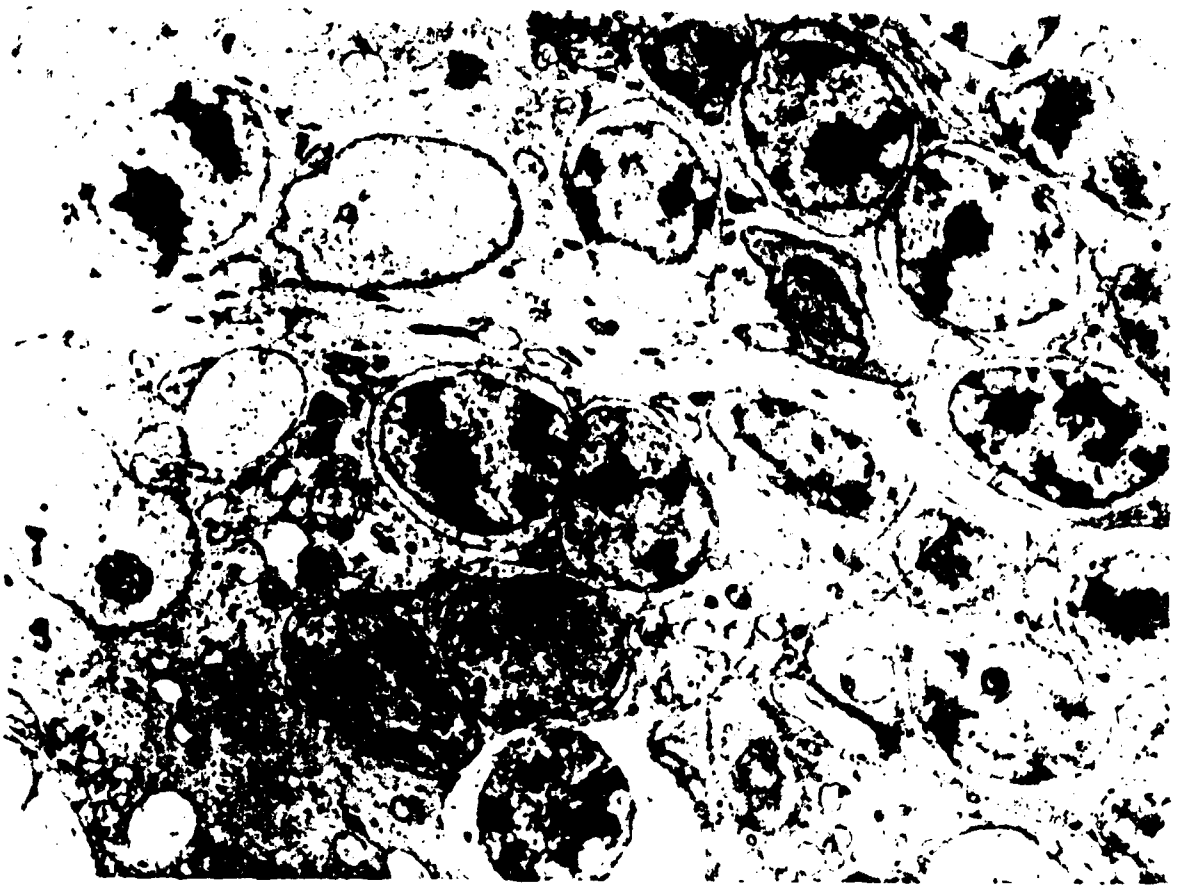
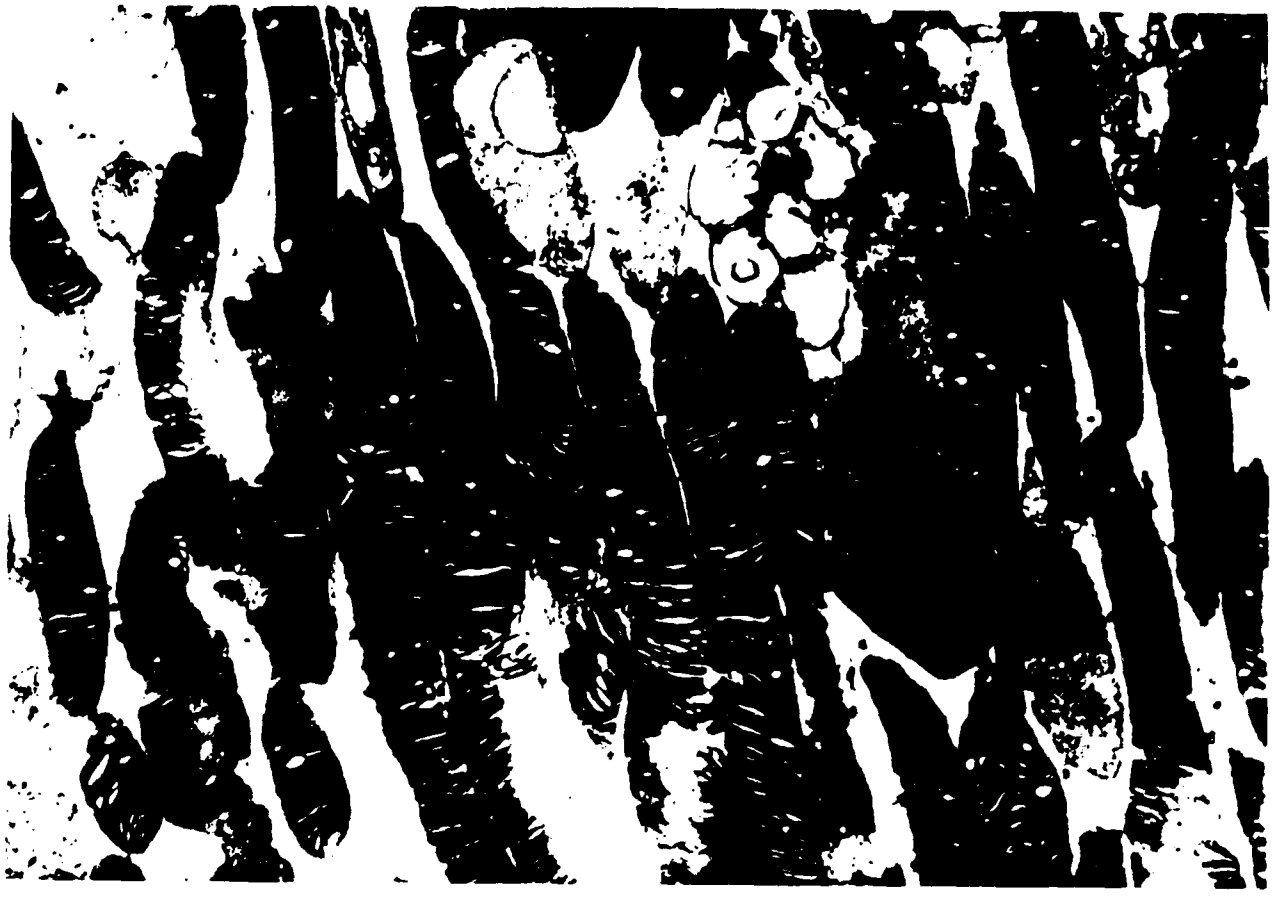


Figure 7a - Electronmicrograph showing intact nuclear layer in a fonte section after PGE1 injection.

Figure 7b



Electronmicrograph showing outer segments following injection with retention of organization.

DISCUSSION

As the subspecialty of vitreoretinal surgery matures, mechanical techniques are reaching their limit. Therefore, pharmacologic therapy to suppress proliferation or reproliferation of epiretinal tissue seems to be a logical next step. We found, however, that some drugs, such as methotrexate, had an expectedly large amount of retinal toxicity even at the level of the outer retina. The other drugs, such as d-penicillamine and colchicine, also showed extensive toxicity. Colchicine, however, appears to be very much dose dependent and as an appealing mechanism in terms of its activity in the cell cycle as a metaphase inhibitor. However, its generalized destruction of microtubular systems makes it a drug that must be approached with a great deal of caution.[1,2,3,4] The complexity of trauma and its testing in a standardized model has been emphasized by Ryan and Cleary.[5,6] The lack of cell cycle synchrony in human ocular trauma cannot be underestimated. For this reason, pharmacologic agents, such as 5-FU to inhibit cell division and PGE1 to inhibit cell contraction, may indeed be the most useful agents of the pharmacologic agents that we tested. However, these drugs are not without their difficulties. First, 5-FU showed a transient corneal haze as we reported in our initial report on clinical examination, however, light microscopy did not show any structural endothelial damage. PGE1 is a very unstable compound and needs precise monitoring of pH to remain present in the eye for a long enough period of time to outlive the active cell cycle of intraocular proliferation. This is an appealing drug, however, since it is naturally occurring and seems to be very well tolerated by the ocular structures. This quality of being naturally occurring, also present with dexamethasone and triamcinolone, is also appealing, however, dexamethasone and triamcinolone have had previous difficulty both in terms of causing retinal necrosis, secondary to carrier activities as observed by other authors, as well as having very variable clinical responses in terms of suppression of tractional retinal detachment.[7,8]

As is true of every study, as the study proceeds, weakness both of the animal model, chosen pharmacologic therapies, as well as new pathogenetic information become available and alter the considerations which seem important.[9,10,11] Our study is no exception to these difficulties. The animal model of the rabbit has advantages in that it is a relatively inexpensive animal model and has been used for a variety of drug testing with seemingly reliable results. However, the animal model does show some differences in terms of the pathogenetic mechanism of tractional retinal detachment between rabbit and human. The vascular distribution of the rabbit with its medullary rays seems to promote posterior proliferation causing tractional retinal detachment in that area. However, in the human eye frequently the most difficult areas of proliferation in retinal traction are in the anterior of the eye. The drug therapy in the rabbit seemed to have its greatest effects posteriorly in the area of the medullary ray. Whether or not concentration was equal in the periphery was unclear from our animal model. Secondly, the agents selected in terms of cellular proliferation were tested in a dose chosen empirically from previous pilot studies in some cases.[12] However, some agents such as colchicine may prove to be more helpful if used in a smaller dose approaching a metaphase inhibitor without the effect of generalized microtubular destruction. This metaphase inhibiting effect may actually be a contractility effect on the microtubular system.

Over the course of this study, new pathogenetic information has become available in terms of the development of proliferative vitreoretinopathy which may be very important in terms of its approach.[13] The concept of hypocellular gel contraction as suggested by Hartzer and Blumenkranz has very wide implications in terms of how trauma and proliferative vitreoretinopathy should be approached. Hypocellular gel contraction would suggest that complete vitrectomy would be very important and that

perhaps there is an ongoing organization cell mediated, but requiring only a few cells, that can organize vitreous collagen, leading to a tractional retinal detachment in areas where collagen is firmly affixed to the retinal surface. This certainly is applicable in trauma since many of these patients are young with incomplete posterior vitreous separations.

There has been work done attempting to stimulate posterior vitreous separation. If the timing of surgical therapy in trauma is important, as suggested by Ryan and Cleary, Coleman and others, it would seem that posterior vitreous separation would be very important. We have thought for a long time of the vitreous as scaffolding for cellular growth, cell-to-cell attachment, cellular contraction and tractional detachment, but it may also be that if collagen organization, based on a few cells activity, is important to tractional detachment, that freeing that vitreous collagen from the retinal surface may be of as great an importance as reducing tractional retinal detachment.[14,15,16] Therefore, pharmacologic agents such as collagenase or early pharmacologic intervention to incapacitate the cell entering the vitreous cavity may be even more important. This might suggest that a pharmacologic bolus of drugs including such things as 5-FU and prostaglandin might also be considered to include an agent which might promote posterior vitreous separation. Prostaglandin PGE1 does not have the usual contractility quality of prostaglandin, which may make it helpful to us in time, but its clinical stability is very variable.[17] PGE1 is currently used by some plastic surgeons to inhibit contracture of tissue and poor skin formation. Collagenase has been suggested as such an agent although the testing on collagenase seems to be very inconclusive at this time.

Certainly, the therapy for trauma and proliferative vitreoretinopathy have progressed together over the last several years. However, trauma, still with its multitude of variables, is one of the most difficult problems to predictably, clinically manage that we face in ophthalmology today. The advent of pharmacologic therapy to suppress intraocular cellular proliferation gives us some hope of combining mechanical and pharmacologic therapy. The goal of mechanical therapy is perhaps to remove an inflammatory milieu and to allow us to gain a somewhat synchronized group of cells within the vitreous cavity that then can be approached in a pharmacologic fashion. Other considerations, such as the effect of a few cells on collagen contracture and retinal vitreal collagen attachments or the production of a reduction of retinal vitreal collagen attachments, either mechanically with vitrectomy or coupled with pharmacologic agents such as collagenase, may play a large role in therapy of trauma.

It appears from our study that there is a difference in efficacy of pharmacologic agents depending on early and late cellular intervention. Late cellular intervention in our study was performed at what was thought to be the mid point (one week) of the suggested two week "life cycle" of the intraocular cellular proliferation. This type of thinking suggests that very early intervention, immediately following trauma with pharmacologic therapy injected either into the vitreous cavity or assuming a breakdown of the ocular blood barrier in the subconjunctival space may be of some benefit in suppressing the immediate cellular proliferative activity and keeping the cell bolus at a manageable level. This type of subconjunctival injection would be minimally risky and has been suggested in the past in human pilot studies. We have used 5-FU in such pilot studies and have found it to be clinically of seemingly little risk to the patient in the subconjunctival space and in a small number of patients have found clinical successes with such problems as giant retinal tears with proliferative vitreoretinopathy.

There certainly is a large amount of work yet still to be done with pharmacologic therapy, but I think that the concept of multiple drug injections analyzing the drug

interactions, the duration of drug in the eye, appropriate carriers for each drug and primate animal models, would be the next step prior to a protocol suggesting human testing of these therapies, especially those which have shown the greatest retinal toxicity.

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